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(54) Title: METHOD OF MEASURING PHYSIOLOGICAL FUNCTION

(57) Abstract

A method of measuring physiological function of a group of body cells, includes the step of selecting a detectable agent capable of emitting a measurable electromagnetic emission. The agent is introduced into body fluid which contacts the group of body cells. The emission is measured, and physiological function is determined based on measurement of the emission.

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METHOD OF MEASURING PHYSIOLOGICAL FUNCTION

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention is in the field of measuring physiological function of a group of body cells.

Description of the Background Art

Current clinical practice for determining liver function includes deriving a CTC score, which is a compilation of laboratory data and clinical assessment of ascites and encephalopathy. D.A. Noe and R.C. Rock (eds), Laboratory Medicine, The Selection and Interpretation of clinical Laboratory Studies, Williams and Wilkins, 1994, Baltimore, MD, Chapter 5, Assessment of Organ Function, by D.A. Noe, p. 55-60, Chapter 19, Liver and Biliary Tract, by A.T. Blei, p. 363-379, Chapter 21, The Kidneys, by D.A. Oken and A.C. Schoolwerth, p. 401-410.

Another test involves the use of indocyanine green (ICG). ICG is known to be exclusively cleared from the bloodstream by the liver. Thus a measurement of the ICG blood clearance time profile is directly related to liver function. J. Caesar, S. Shaldon, L. Chiandussi, L. Guevara, and Sheila

Shaldon, L. Chiandussi, L. Guevara, and Sheila Sherlock, "The use of indocyanine green in the measurement of hepatic blood flow and as a test of hepatic function," *Clin. Sci.* **21**, 43-57 (1961).

The ICG test has undergone an evolution in technology. In its first incarnation, blood was withdrawn from the subject at several times following an IV bolus injection. The blood samples were then processed spectrophotometrically to determine ICG concentration. R. Jalan and P. C. Hayes, "Review article: quantitative tests of liver function," *Aliment Pharmacol. Ther.* **9**, 263-270 (1995); A. W. Hemming, C. H. Scudamore, C. R. Shackleton, M. Pudek, and S. R. Erb, "Indocyanine green clearance as a predictor of successful hepatic resection in cirrhotic patients," *Am. J. Surg.* **163**, 515-518 (1992); P. Ott, S. Keiding, and L. Bass, "Plasma elimination of indocyanine green in the intact pig after bolus injection and during constant infusion: comparison of spectrophotometry and high-pressure liquid chromatography for concentration analysis," *Hepatology* **18**, 1504-1515 (1993). Subsequently, a non-invasive technique employing ear densitometry was developed. C. M. Leevy, F. Smith, J. Longueville, G. Paumgartner, and M. M. Howard, "Indocyanine green clearance as a test for hepatic function: Evaluation by dichromatic ear densitometry," *Journal of Medicine* **24**, 10-27 (1993). Problems associated with the clinical development of this device recently led Japanese researchers to improve upon the ear densitometry technique. This newer method, termed the finger-piece method, employs transmitted light of two wavelengths measured throughout a finger to deduce ICG concentration. M. Kanda, K. Taniguchi, K.

Awazu, Y. Ishigami, M. Masuzawa, and H. Abe, "Continuous monitoring of Cardiogreen removal by a diseased liver using an optical sensor," *Proc. SPIE* **904**, 39-46 (1988); M. Nakayama, N. Kanaya, S. Fujita, and A. Namiki, "Effects of ephedrine on indocyanine green clearance during spinal anesthesia: Evaluation by the finger piece method," *Anesth. Analg.* **77**, 947-949 (1993); N. Kanaya, H. Iwasaki, and A. Namiki, "Noninvasive ICG clearance test for estimating hepatic blood flow during halothane and isoflurane anaesthesia," *Can. J. Anaesth.* **42**, 209-212 (1995); N. Kanaya, M. Nakayama, S. Fujita, and A. Namiki, "Comparison of the effects of sevoflurane, isoflurane and halothane on indocyanine green clearance," *Br. J. Anaesth.* **74**, 164-167 (1995); S. Shimizu, W. Kamiike, N. Hatanaka, Y. Yoshida, K. Tagawa, M. Miyata, and H. Matsuda, "New method for measuring ICG Rmax with a clearance meter," *World J. Surg.* **19**, 113-118 (1995).

Both ear densitometry and the finger-piece method involve measuring absorption (or transmission) of light by ICG.

Also of interest is that *in vitro* fluorometric determination of ICG in plasma has been demonstrated, B. Hollins, B. Noe, and J.M. Henderson, "Fluorometric determination of indocyanine green in plasma," *Clin. Chem.* **33**, 765-768 (1987).

Other references of general interest include: R.L. Sheridan, et al., "Burn depth estimation by indocyanine green fluorescence: Initial human trial," *Journal of Burn Care & Rehabilitation* **16**, 602-604 (1995); M.A. O'Leary, D.A. Boas, B. Chance, and A.G Yodh, "Reradiation and imaging of diffuse photon

density waves using fluorescent inhomogeneities," *Journal of Luminescence* **60 & 61**, 281-286 (1994); X. Li, B. Beauvoit, R. White, S. Nioka, B. Chance, and A. Yodh, "Tumor localization using fluorescence of indocyanine green (ICG) in rat models," *Proc. SPIE* **2389**, 789-797 (1995).

There remains a need in the art for improved methods of measuring physiological function.

SUMMARY OF THE INVENTION

In accordance with the present invention, a method of measuring physiological function of a group of body cells, includes the step of selecting a detectable agent capable of emitting a measurable member comprising an electromagnetic emission. The agent is introduced into body fluid which contacts the group of body cells. The emission is measured, and physiological function is determined based on measurement of the emission.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic illustration of an *in vivo* fluorescence detection apparatus in accordance with one embodiment.

FIG. 2 graphically depicts the *in vivo* fluorescence time dependence after a bolus injection of FITC labeled poly-d-lysine in a single rat pre-kidney ligation (Normal) and post-kidney ligation (Ligated). The solid line is a single exponential fit to the measured data. (Excitation at 488 nm, emission monitored at 518 nm.)

FIG. 3 graphically depicts *in vivo* fluorescence time dependence for three rats after a bolus

injection of ICG. The solid lines are single exponential fits to the measured data.

FIG. 4 graphically depicts the *in vivo* fluorescence time dependence of a succession of bolus injections in one rat. In chronological order: ICG (ICG-1), FITC only, saline only, ICG again (ICG-2).

FIG. 5 graphically depicts the *in vivo* fluorescence time dependence after a bolus injection of ICG in a single rat pre-partial liver ablation (Normal) and post-partial liver ablation (Ablated). The solid lines are single exponential fits to the measured data.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with one embodiment of the present invention, a method is disclosed for determining cell and/or organ function by measuring the blood pool clearance of a targeted agent, sometimes referred to herein as tracer. The cell and/or organ function can be determined by the rate these cells remove the tracer from the bloodstream. Function can also be assessed by measuring the rate the cells of interest accumulate the tracer or convert it into an active or other form.

The agent may be targeted to a group of cells or organ which is a high capacity clearance system. The agent may contain a chromophore and/or fluorophore.

For agents containing chromophores and/or fluorophores, blood pool clearance may be measured using a light source/photocell device that measures tissue absorbance or fluorescence in a non-target site, such as an ear lobe, finger, brain or retina. Accumulation of the tracer within the cells of interest can be assessed in a similar fashion. The detection of such accumulation can be facilitated by

using fluorophores which emit in the near infrared wavelengths since body tissues are relatively transparent at these wavelengths.

The Agent may be introduced into the patient by any suitable method, including intravenous, intraperitoneal or subcutaneous injection or infusion, oral administration, transdermal absorption through the skin, or by inhalation.

The present invention may be used for rapid bedside evaluation of biologic functions. For example, data on cardiac output, cause of hypercholesterolemia, as well as renal and hepatic function, may be obtained in less than sixty minutes at the bedside after a single intravenous injection. In accordance with one embodiment, a patient may receive a bolus injection of a plurality (e.g., three) different compounds, each containing a different agent (e.g., fluorophore).

Cardiac output may be determined utilizing the present invention in conjunction with known methods such as the Fick principle.

Glomerular filtration may be determined by clearance of a low molecular weight fluorescent agent such as fluorescein-poly-D-lysine or fluorescein-inulin.

Whether hypercholesterolemia is caused by poor LDL clearance may be determined by analyzing the clearance of fluorescent- labeled LDL. Hepatic function may be as assessed by measuring the clearance rate of a fluorescent-labeled asiaglycoprotein or a dye such as indocyanine green.

The present invention includes fluorescence detection of an agent which is cleared from the bloodstream by the kidneys. Thus, assessment of renal function by *in vivo* fluorescence detection is

encompassed within the invention. The invention can also be used to monitor the efficiency of hemodialysis.

Tumor cells or brain cells also can be targeted in accordance with the invention.

The clearance of a plurality of separate tracers may be determined simultaneously by selecting excitation wavelengths and filters for the emitted photons. The concentration/time curves may be analyzed in real time by a microprocessor. The resulting clearance rates may be calculated and displayed for immediate clinical impact. In cases where unlabeled competing compounds are present (e.g., LDL, asialoglycoproteins), a single blood sample may be analyzed for the concentration of these competing compounds and the results used to calculate a flux (micromoles/minute) through the clearance pathways.

In order to demonstrate utility of the invention, a non-invasive fluorescence detection system in accordance with the present invention was employed to continuously monitor dye removal from the vasculature. Differentiation between normal and abnormal organ function in a rat model was demonstrated for both liver and kidney. With reference to Figure 1, a fiber optic 10 transmited light from source 12 to ear 14. A second fiber optic 16 positioned near the ear 14 transmited the fluorescent light to a detector system 20. Two dyes were employed in this initial study. Indocyanine green is exclusively cleared from the blood stream by the liver, and was excited *in vivo* with laser light at 780 nm. The fluorescence signal was detected at 830 nm. A characteristic clearance curve of normal hepatic function was obtained. Upon ablation of a

portion of the liver, the clearance curve was greatly extended as expected. FITC labeled, succinylated poly-d-lysine was excited *in vivo* with laser light at 488 nm. The fluorescence signal was detected at 518 nm. A characteristic clearance curve of normal renal function was obtained. Upon ligation of both kidneys, the clearance curve remained elevated and constant, indicating little if any clearance. See Figure 2.

With the schematic apparatus for non-invasive *in vivo* detection of fluorescence shown in Figure 1, for ICG fluorescence detection, a nominal 785 nm collimated solid state laser source was employed (LaserMax Inc. model # LAS-300-780-5). For FITC fluorescence detection, an argon ion laser (Coherent Innova model 90) tuned to the 488 nm line was used. Either laser source was directed into the end of a 3.2 mm inner diameter glass fiber optic bundle 10 (Oriel #77526). The other end of this laser delivery bundle was placed approximately 2 cm from the rat ear 14 at an approximate 45° angle. A second similar fiber optic bundle 16 for use as the fluorescence detection conduit was placed approximately 1 cm from the ear 14 at a 30° angle.

The exit end of the detection fiber bundle 16 was positioned at the focal length of a 20 mm focal length lens 18. The output light was thus collimated after exiting the bundle and passing through the lens. A narrow band interference filter 20 (IF) was the next element in the optics train (CVI Laser Corporation), allowing light of the appropriate wavelength to pass on to the detector 20. For the ICG fluorescence experiment, an 830 nm filter (10 nm FWHM) was used. For the FITC fluorescence experiment, a 518 nm filter (3 nm FWHM) was used.

The detector 20 was a small silicon photodiode (UDT model PIN-10D) connected to a transimpedance amplifier (UDT model 101C). A digital voltmeter 22 (DVM) monitors the output signal. A subsequent voltage amplifier 24 (Tektronix AM-502) boosts the signal if needed. The amplifier output is connected to a National Instruments BNC-2080 breakout board, which is interfaced to a National Instruments DAQ-700 data acquisition board 26 (A/D). LabVIEW® data acquisition software in computer 28 collects the experimental raw data.

The current method contrasts with the prior art methods which used radiolabeled tracers. The present method eliminates concerns about radioactivity and allows concurrent measurements of different parameters simply by rapid alteration of the excitation and emission wavelengths.

The invention is further illustrated by the following examples, which are not intended to be limiting.

<u>Example 1</u>:

For these in-vivo studies, normal Sprague-Dawley rats weighing ~250 grams were first anesthetized with urethane (1.35g/kg) administered via intraperitoneal injection.

After each animal had achieved the desired plane of anesthesia, a small (0.5cm) incision was made in the upper thorax exposing the left jugular vein. The lobe of the left ear was fixed to a glass microscope slide, and the incident laser light delivered from the fiber optic was centered on that ear. Data acquisition was then initiated, and a background reading of fluorescence was obtained prior to administration of the test article. Next, the dye (ICG for liver clearance assessment, FITC labeled

poly-d-lysine for kidney clearance assessment) was administered via the jugular vein. The fluorescence signal immediately increased to a peak value. The signal decayed as a function of time as the dye presumably cleared from the bloodstream.

The anesthetized rat was placed on its back and a midline ventral abdominal skin incision made extending from the xiphoid cartilage to approximately midway to the tail. A similar incision was then made in the abdominal muscles exposing the liver. The rat was repositioned and a bolster placed under the thorax to cause the liver to fall slightly forward and away from the diaphragm. The median and left lateral lobes of the liver were gently moved out of the abdominal cavity and placed onto a gauze pad wetted with saline. The two lobes were vertically raised and a 3-0 ligature placed around the isolated lobes but were not ligated at this point of the procedure. The lobes were replaced in the abdominal cavity and the bolster removed. The incision was closed with wound clips.

ICG was administered to the animal via the exposed jugular vein as in the previous study. The clearance of the test article was monitored as before to determine the normal hepatic clearance of the ICG. After the normal clearance curve was obtained, the ligature around the two isolated lobes of the liver was tied securely to effect a partial hepatectomy. The animal was allowed to equilibrate for 20 minutes in this state. The ICG was next administered via the exposed jugular vein, and the clearance of the test article monitored. Clearance curves of normal versus partial hepatectomized animals were obtained for an n=3 sample.

The time dependence of fluorescence measured at the ear pre and post bolus injection of ICG for three rats is shown in Figure 3. The data can be described in terms of three stages. Stage 1 consisted of approximately the first 30 seconds of data, which was gathered pre-bolus injection. These data were constant and represented the baseline value for the forthcoming experiment. The value of the baseline should be zero, since no fluorescence is occurring during this stage. Stage 2 occurred several seconds post-injection, the signal rapidly rose to a maximum as the dye reached the ear and equilibrated in the blood pool. In the third stage, the fluorescence signal decayed with time as the liver filtered the ICG out of the blood stream. Visually, the decay rates were similar for all three. After 15 minutes, approximately 90% of the initial signal was lost.

To verify that the measurement was indeed that of ICG fluorescence, the following control study was performed. A rat was injected, as above, with 500 µL of 1.41 mM ICG. A normal fluorescence time course was obtained and is labeled as ICG-1 in Figure 4. Then the same rat was injected with 500 µL of 1.41 mM fluorescein (Sigma, St. Louis, MO). As shown in Figure 4, no fluorescence signal was detected. As a further check, 500 µL of saline solution (Baxter, Deerfield, IL) was injected into the same rat next. Again, no detectible signal was obtained. Finally, the rat was once again injected with 500 µL of 1.41 mM ICG, and a second "normal" curve was obtained.

To verify that these fluorescence decay curves were related to liver function, an experiment involving a partial liver ablation was performed. The partial liver ablation procedure is outlined above. Once the surgery was complete, and the

ligatures for use in partially ablating the liver were ready, the rat was injected with 500 µL of 1 mM ICG solution. A normal fluorescence time course curve was obtained and is shown in Figure 5.

The liver was then partially ablated by tightening the ligatures. The rat was allowed to equilibrate for ten minutes. Next, another injection of 500 µL of 1mM ICG was given. The fluorescence time curve was measured and is also shown in Figure 5. The capability of the liver to remove ICG from the blood pool was drastically altered, the fluorescence decay rate for the partially ablated liver was much slower than the normal. Upon sacrifice, the liver was weighed and 44% of the liver was found to be ablated.

Example 2:

500 mg (~125 µmole equivalent Lysine) poly-d-lysine 4000 (Sigma P-0296) was dissolved in 10 ml 0.1 M Na_2CO_3, in a dark glass vial with magnetic stirring bar. 24.3 mg (62.5 µmoles) FITC (Fluorescein isothiocyanate, Sigma F-7250) was dissolved in 1.5 ml DMSO (dimethyl sulfoxide). At 25°C, with stirring, the FITC solution was slowly added to the poly-d-lysine solution. The reaction was allowed to proceed for 30 minutes at 25°C, then transferred to 4°C, and stirred 12 hrs. The fluorescein conjugate was separated from unbound FITC by gel filtration on Sephadex G-25, eluting with 0.9% (w/v) NaCl.

20 ml 0.9% NaCl containing ~30 µmoles of the above conjugate were placed in a dark glass vial with magnetic stirring bar and pH electrode at 25°C; pH was raised to 9.5 by addition of 0.5 M NaOH. 500 mg of succinic anhydride was added slowly, with stirring, to this solution over a period of 30 minutes, maintaining pH 9.5-10.0 by the addition of 0.5 M NaOH. The pH was then allowed to fall to a

stable value of 7.5, with a final volume of 27 ml. The reaction mixture was dialyzed vs. 0.9% (w/v) NaCl using a dialysis membrane with 3.5 kd cutoff, and the retained polymer conjugate, at ~4.0 µM fluorescein concentration equivalent, was used directly for infusion.

The anesthetized rat was placed on the ventral surface and bilateral dorsoventral incisions were made in the abdominal cavity near to the coastal border of the thorax. The kidneys were freed of connective tissue and were gently pulled away from the abdomen by grasping the perirenal fat tissue. A single 3-0 ligature was placed around the renal vessels and ureter so as not to occlude collateral vessels. The ligatures were not tied at this point of the procedure.

Succinylated, fluorescein-labeled poly-d-lysine was administered via the exposed jugular vein. The clearance of the test article was monitored as before to determine the normal renal clearance of poly-d-lysine. After the normal clearance curve was obtained, the ligature was tied to effect a total (bilateral) nephrectomy. The animal was allowed to stabilize in this condition for 20 minutes. The test article was next administered via the exposed jugular vein and the clearance of the compound monitored. Clearance curves of normal versus total nephrectomized animals were obtained for an n=3 sample.

The utility of non-invasive fluorescence detection to monitor liver or kidney function has been established.

The steps of the invention may be repeated in order to determine if physiological function is changing.

Indocyanine green is a dye which fluoresces at a wavelength of about 830 nm and was used to measure the physiological function of the liver. In order to measure the physiological function of the liver, a body portion was irradiated with light with a wavelength of about 780 nm. The physiological or hepatic function of liver cells was measured using the claimed method.

Fluorescein labeled, succinylated poly-d-lysine is a dye which fluoresces at a wavelength of about 518 nm and was used to measure the physiological function of the kidneys. In order to measure the physiological function of the kidneys, a body portion was irradiated with light with a wavelength of about 488 nm. Renal function was measured using the above-described method of the invention. See Figure 2.

The dyes were intravenously injected. A body portion, which included blood vessels near the surface of the skin, was irradiated with a laser or with infrared radiation.

The claimed invention may also be used to evaluate hypercholesterolemia. Clearance rate measurements may allow the clinician to determine whether high serum cholesterol resulted from increased rate of LDL production or from decreased rate of LDL clearance, which may impact therapy. The claimed invention may also be used to measure cardiac output. The ability to concurrently measure cardiac function while also measuring hepatic and renal function may allow the clinician to draw preliminary conclusions about whether any observed changes in hepatic and renal functions were due to primary renal or hepatic disease or secondary to heart disease.

Since many modifications, variations and changes in detail may be made to the described embodiments,

it is intended that all matter in the foregoing description and shown in the accompanying drawings be interpreted as illustrative and not in a limiting sense.

CLAIMS

1. A method of measuring physiological function of a group of body cells comprising the steps of:

a) selecting a detectable agent capable of emitting a measurable member comprising an electromagnetic emission, said agent being selectively removed from a body fluid by said group of body cells, and wherein said emission occurs in said body fluid;

b) introducing said agent into said body fluid of a patient, which body fluid contacts said group of body cells, and wherein said emission occurs in said body fluid;

c) measuring said emission from a body portion through which said body fluid passes; and

d) determining said physiological function based on measurement of said emission.

2. A method as defined by claim 1, wherein steps b) through d) are repeated to determine if physiological function changes.

3. A method as defined by claim 1, wherein said agent is injected.

4. A method as defined by claim 1, wherein said agent is intravenously injected.

5. A method as defined by claim 1, wherein said body portion includes blood vessels near a surface of skin of said patient.

6. A method as defined by claim 1, wherein said agent comprises a fluorophore or chromophore.

7. A method as defined by claim 6, wherein said agent comprises a fluorophore.

8. A method as defined by claim 1,

wherein said agent is a dye capable of fluorescing at a first wavelength upon being irradiated with light of a second wavelength;

wherein prior to said measuring step, said body portion through which said body fluid passes is irradiated with said light of said second wavelength, causing said dye to fluoresce at said first wavelength;

wherein said emission being measured is fluorescence of said dye at said first wavelength; and

wherein said determination of physiological function is based on measurement of fluorescence of said first wavelength.

9. A method as defined by claim 8, wherein the steps of introducing, irradiating, measuring, and determining are repeated to determine if physiological function changes.

10. A method as defined by claim 8, wherein said dye is indocyanine green.

11. A method as defined by claim 8, wherein said dye is fluorescein labeled, succinylated poly-d-lysine.

12. A method as defined by claim 8, wherein said second wavelength is about 400 - 1200 nanometers.

13. A method as defined by claim 8, wherein said second wavelength is about 488 nanometers.

14. A method as defined by claim 8, wherein said body portion is irradiated with a laser.

15. A method as defined by claim 8, wherein said body portion is irradiated with infrared radiation.

16. A method as defined by claim 8, wherein said body cells are kidney cells.

17. A method as defined by claim 8, wherein said body cells are liver cells.

18. A method as defined by claim 1, wherein said body cells are heart cells.

19. A method as defined by claim 8, wherein said first wavelength is about 830 nanometers.

20. A method as defined in claim 8, wherein said first wavelength is about 518 nanometers.

21. A method as defined by claim 8, wherein said physiological function is renal function.

22. A method as defined by claim 8, wherein said physiological function is hepatic function.

23. A method as defined by claim 1, wherein said physiological function is cardiac function.

24. A method as defined in claim 1, wherein said cells are tumor cells.

25. A method as defined in claim 1, wherein said cells are brain cells.

FIG. I

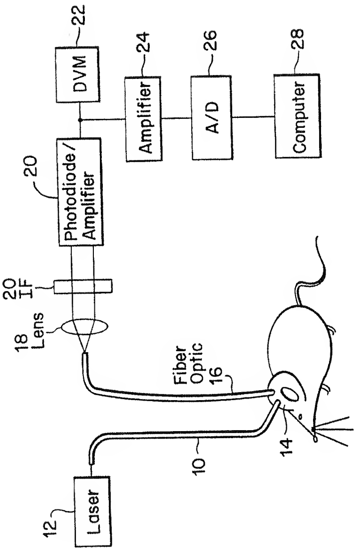

22
DVM
24
Amplifier
26
A/D
28
Computer
20
Photodiode / Amplifier
20
IF
18
Lens
Fiber
Optic
16
10
14
12
Laser

FIG. 2

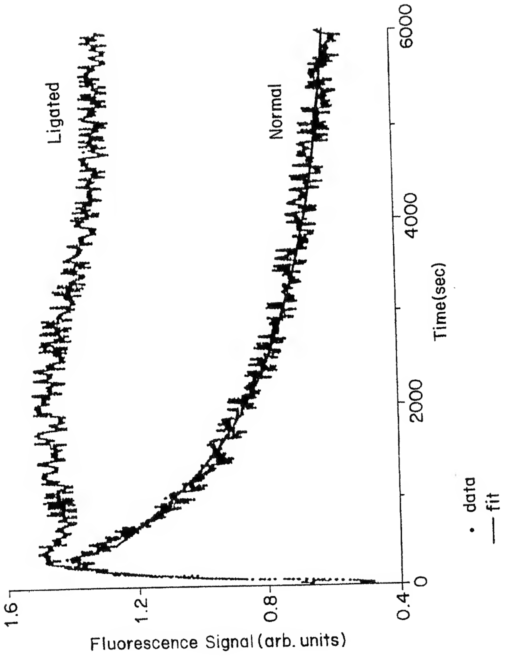

Ligated
Normal
Fluorescence Signal (arb. units)
1.6
1.2
0.8
0.4
0
2000
4000
6000
Time(sec)
• data
— fit

FIG. 3

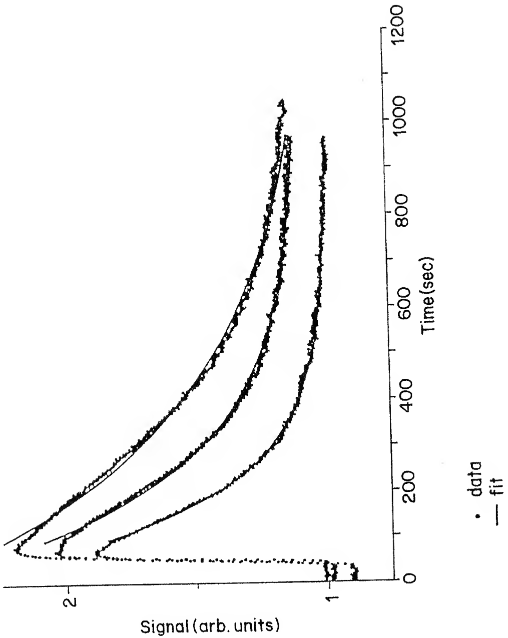

0
200
400
600
800
1000
1200
Time(sec)
2
1
Signal (arb. units)
• data
— fit

FIG. 4

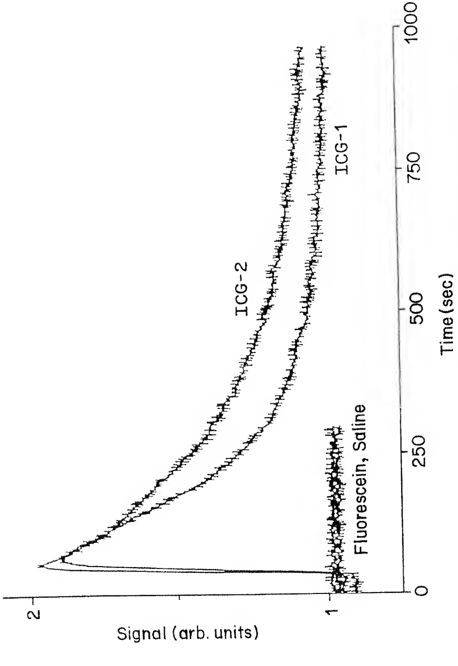
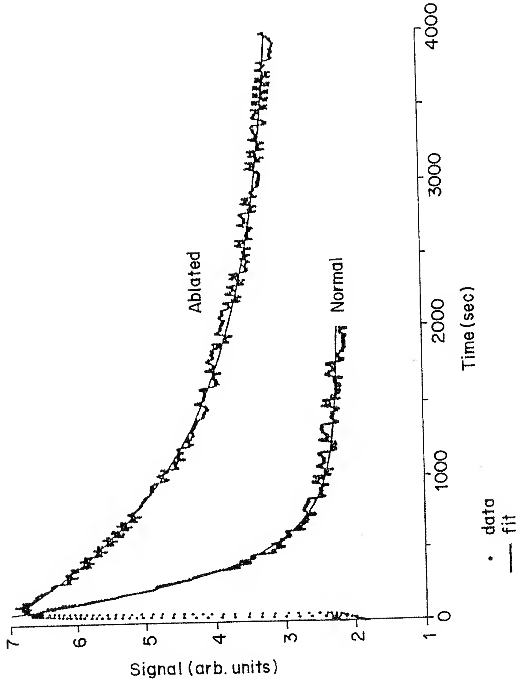

ICG-2
ICG-1
Fluorescein, Saline
Signal (arb. units)
2
1
0
250
500
750
1000
Time (sec)

FIG. 5


Ablated
Normal
data
fit
Signal (arb. units)
Time (sec)
1
2
3
4
5
6
7
0
1000
2000
3000
4000

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 98/01624

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K49/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SOULIE, S. ET AL: "In vivo pharmacokinetic study of two fluorescein derivatives by fluorescence spectroscopy." PROC. SPIE-INT. SOC. OPT. ENG. (1995), VOLUME DATE 1995, 2627, 109-17 CODEN: PSISDG;ISSN: 0277-786X, 1995, XP002071643 see page 111 ---	

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 July 1998	31.07.98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Berte, M

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE BIOSIS BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US SCOTT V L ET AL: "Comparison of indocyanine green clearance using serum spectrophotometric analysis and a non-invasive pulse-spectrometry probe in patients with liver failure." XP002071647 see abstract & ANNUAL MEETING OF THE AMERICAN SOCIETY OF ANESTHESIOLOGISTS, SAN DIEGO, CALIFORNIA, USA, OCTOBER 18-22, 1997. ANESTHESIOLOGY (HAGERSTOWN) 87 (3 SUPPL.). 1997. A77. ISSN: 0003-3022, ---	1-10, 12-15, 17,19, 20,22,24
X	DATABASE CHEMABS CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US AWAZU, KUNIO ET AL: "The data processing function of the ICG clearance meter" XP002071648 see abstract & YAKURI TO CHIRYO (1992), 20(SUPPL. 10), S2427-S2431 CODEN: YACHDS;ISSN: 0386-3603, 1992, ---	1-10, 12-15, 17,19, 20,22,24
X	SHINOHARA, HISASHI ET AL: "Direct measurement of hepatic indocyanine green clearance with near-infrared spectroscopy: Separate evaluation of uptake and removal" HEPATOLOGY (PHILADELPHIA) (1996), 23(1), 137-44 CODEN: HPTLD9;ISSN: 0270-9139, 1996, XP002071644 see page 143, column 1, paragraph 3 - column 2, paragraph 3 ---	1-10, 12-15, 17,19, 20,22,24
X	MORDON, SERGE ET AL: "Fluorescence measurement of diode (805nm) laser-induced release of 5,6-CF from DSPC liposomes for monitoring of temperature: An in-vivo study in rat liver using indocyanine green potentiation" PROC. SPIE-INT. SOC. OPT. ENG. (1995), 2391(LASER-TISSUE INTERACTION VI), 475-83 CODEN: PSISDG;ISSN: 0277-786X, 1995, XP002071645 see abstract see page 477; figure 2 ---	1-10, 12-15, 17,19, 20,22,24
Y	WO 97 06829 A (UNIV BIRMINGHAM ;MILLS CHARLES OSWALD (GB)) 27 February 1997 see page 1, paragraph 2 - paragraph 3; claims --- -/--	1-25

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4 848 349 A (SHERMAN IGOR A ET AL) 18 July 1989 see column 1, line 43 - line 59 see column 3, line 5 - line 22	1-25
X	see column 6, line 18 - line 50; claims	1-25
X	DATABASE EMBASE ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL AN=96333069, XP002071649 see abstract & TSAI K.N. ET AL.: "Comparison of ICG Finger Monitor system with conventional blood sampling ICG clearnace test in patients with acute sever hepatitis." GASTROENTEROLOGICAL JOURNAL OF TAIWAN, vol. 13, no. 2, 1996, pages 186-193,	1-10, 12-15, 17,19, 20,22,24

INTERNATIONAL SEARCH REPORT	International application No. PCT/US 98/01624

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-25
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 1-25
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searcheble claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte   ional Application No
PCT/US 98/01624

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9706829 A	27-02-1997	EP 0844890 A	03-06-1998
US 4848349 A	18-07-1989	NONE	